Miniprep (Qiageen Kit)

All centrifugation steps are carried out at 17.900 g in a conventional table-top microcentrifuge if not stated differently. Prewarm elution buffer to 70 – 80 °C.

1. Pellet **5 ml** bacterial overnight culture in a 2 ml microcentrifuge tube by centrifugation at 6800 g for 3 min (**or at 17.900 g for 1 min each**)
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue reagent, the solution will trn colorless
5. Centrifuge for 3-30 min in a table-top microcentrifuge (**Centrifuge until** cell debris pellet is properly seperated from the supernatant)
6. Apply 800 µl supernatant from step 5 to QIAprep 2.0 spin column by pipetting. (**If possible, use more than 800 µl, as long as no protein is pipetted**)
7. Centrifuge for 30 s and discard the flow-through.
8. Wash the spin column by adding 500 µl ml Buffer PB. Centrifuge for 30 s and discard the flow-through
9. Wash the spin column by adding 750 µl Buffer PE. Centrifuge for 30 s and discard the flow-trough
10. Centrifuge for 60 s to remove residual wash buffer. Place spin column in microcentrifuge tube into thermo mixer at 70-80 °C until ethanol smell has vanished.
11. Place the spin column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 30-50 µl prewarmed Buffer EB or water to the center of the spin column. Let stand for 1 min, then centrifuge for 1 min.
12. **Pipette the elution into the center of the spin column and centrifuge again for 1 min.**